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Review

Host proteins involved in HIV infection: New therapeutic targets

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ABSTRACT

Current treatment of HIV/AIDS consists of a combination of three to five agents targeting different viral proteins, i.e. the reverse transcriptase, protease, integrase and envelope, and aims to suppress viral replication below detectable levels. This "highly active antiretroviral therapy" (HAART) has brought an enormous benefit for life expectancy and quality in HIV-1-infected individuals, at least in industrialized countries. However, significant limitations with regard to efficiency, drug resistance, side effect and costs still exist. Recent data suggest that cellular factors also represent useful targets for therapy. Here, we summarize findings from several genome-wide screens that identified a large number of cellular factors exploited by HIV-1 at each step of its life cycle. Furthermore, we discuss the evidence that humans are equipped with powerful intrinsic defense mechanisms against retroviruses but that HIV-1 has evolved elaborate ways to counteract or evade them. Preventing the use of host cell proteins obligatory for viral replication or strengthening the cellular defense mechanisms may help to reduce viral replication to harmless levels. A better understanding of the host factors that promote or restrict HIV-1 replication may thus lead to the development of novel therapeutics against HIV/AIDS.

1. Introduction

Currently, more than 25 antiretroviral drugs are available to treat human immunodeficiency virus (HIV) infection. The great majority of them target the HIV-1 reverse transcriptase (RT) (nucleoside and nucleotide RT inhibitors: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine and tenofovir and non-nucleoside RT inhibitors: nevirapine, delavirdine, efavirenz and etravirine) and the viral protease (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir and darunavir) (Fig. 1). More recently, antiretroviral drugs that inhibit the viral integrase (IN) (raltegravir) or the six-helix bundle core formation of the gp41 transmembrane protein required for virus–cell fusion (enfuvirtide) have been approved for the clinic [1]. While this growing repertoire of antiretroviral agents is impressive, none of these drugs is useful for the treatment of HIV/acquired immunodeficiency syndrome (AIDS) on its own because HIV-1 is highly variable and capable of developing resistance against all of them. This high genetic variability provided the rationale for the development of "highly active antiretroviral therapy" (HAART) consisting of combinations of three or more antiretroviral agents. HAART aims to suppress viral replication to such low levels that the emergence of drug-resistant HIV-1 variants is prevented. Furthermore, while the diverse HIV-1 species (called quasi-species) found in a single individual will most likely already contain the genetic changes reducing its susceptibility to single drugs, it is more difficult for the virus (and sometimes associated with decreased "fitness") to acquire the complex combination of mutations required for multi-drug resistance.

Treatment of HIV-1-infected individuals by HAART usually allows to reduce the plasma viral load to undetectable levels, improves CD4+ T cell counts, delays disease progression and promotes survival. However, although the development of HAART was certainly the greatest success of AIDS research and allows the reduction of morbidity and mortality wherever it is available, it also has major limitations. Furthermore, HAART is expensive and requires an infrastructure with a functional health care system allowing the medical monitoring of the success of antiretroviral therapy to prevent or at least delay the emergence of drug-resistant HIV-1 strains. The vast majority of ~34 million people currently infected with HIV-1 live in developing countries and – although access to antiretroviral drugs in the developing world is improving – most of them still do not have access to antiretroviral therapy [2,3]. Thus, while HAART constitutes an effective approach for the treatment of AIDS and also prevents HIV-1 transmission by reducing the viral loads, it still has little impact on the global spread of the virus (~2.5 million new HIV infections per year) and the global number of fatalities caused by AIDS (~2 million per year). Life expectancy has drastically fallen in some countries that are most severely affected by HIV/AIDS, e.g. in Zimbabwe it is only 34 years for women and 37 years for men [2]. Even under optimal conditions, HAART has significant drawbacks, e.g. it is frequently associated with significant side effects (such as metabolic and cardiovascular disorders), with immune reconstitution disease, and with the development of resistant HIV-1 strains (particularly in the

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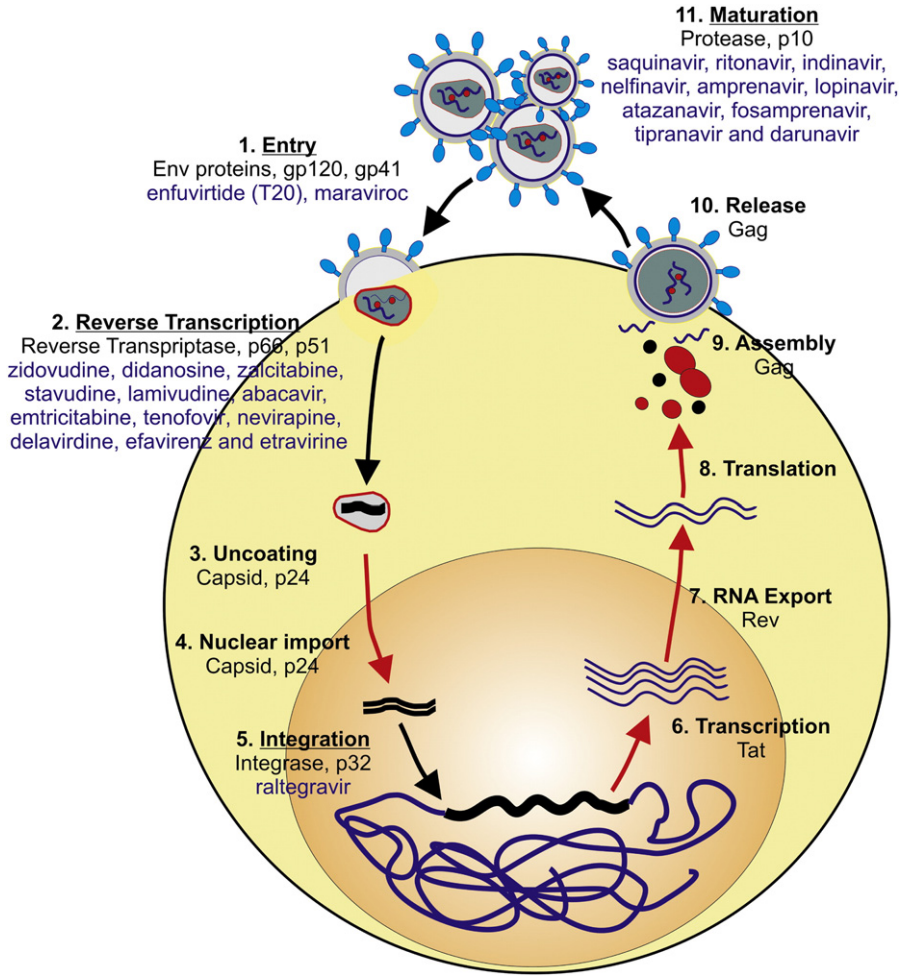

Fig. 1. Viral replication cycle and the steps currently inhibited by HAART. Viral proteins involved in the individual steps are indicated in black and antiretroviral agents blocking them in blue.

case of patients' non-adherence to treatment). Furthermore, HAART requires life-long daily treatment because it does not allow to eliminate resting long-lived cells containing integrated proviruses and thus fails to eradicate the virus entirely.

Because of the drawbacks of current combination antiretroviral therapy, it remains a major interest to develop new antiretroviral drugs or innovative therapies to reduce undesired side effects, to prevent the emergence of drug resistance or even to attack the viral reservoirs [4,5]. Indeed, a major barrier to curing HIV infection remains the ability of HIV to integrate in the host genome and remain latent. The thus-generated viral reservoirs cause viral rebound upon HAART interruption and impose lifelong antiretroviral therapy with its many associated side effects and possible development of resistance. Here, we focus on approaches aiming to target cellular rather than viral proteins. It is long known that HIV-1 utilizes host factors at many steps of its life cycle. However, currently only a single drug targeting a cellular protein has been approved for the clinic: Maraviroc binds to the HIV-1 entry cofactor CCR5 and blocks its interaction with the viral envelope gp120 to prevent the membrane fusion events necessary for viral entry [6]. Recent studies using genome-wide screening technologies have identified large numbers of host factors that may be required for virus replication and thus represent potential therapeutic targets [7–9]. Another recent development is the realization that humans are equipped with factors that directly inhibit retroviruses [10,11]. For example, tripartite motif 5-alpha (TRIM5α) proteins can block incoming retroviral capsids in a species-specific manner [12] and the cellular apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3 (APOBEC3) cytidine deaminase induces lethal hypermutation of the viral genome [13]. Finally, the recently discovered restriction factor "tetherin" (also called BST-2, HM1.24 or CD317) inhibits HIV-1 particle release [14,15]. These ancient antiviral defense mechanisms were obviously quite successful in past encounters with retroviruses since about 8% of the human genome is of retroviral origin [16]. HIV-1, however, has evolved the ability to counteract these by several "accessory" viral proteins (Viral infectivity factor (Vif), viral protein U (Vpu)) or can avoid them by its high variability and is thus capable to replicate efficiently in the hostile environment of the human cell [10,11]. Strengthening these cellular defense mechanisms or weakening the viral antagonists may help to control HIV-1 replication.

2. Host factors as targets for antiretroviral therapy

2.1. Targeting viral entry

All viruses are obligate intracellular parasites without an independent metabolism and thus strictly dependent on their target cells for reproduction. The first cellular factor shown to be required for HIV-1 replication is the primary viral CD4 receptor that has been discovered more than two decades ago and determines the viral tropism for CD4+ T cells and tissue macrophages [17]. A variety of strategies has been pursued to block the interaction between the external viral envelope glycoprotein gp120 and CD4. These include soluble CD4 or CD4 mimics that seem to induce a non-functional conformation of gp120, small molecule inhibitors that target the

conserved CD4-gp120 binding site as well as CD4 antibodies [18]. One CD4 antibody (TNX-355) has shown some beneficial effects in clinical studies [19] and seems to act synergistically with enfuvirtide (T20) that interacts with gp41 and blocks a later step in the viral entry process [20]. However, TNX-355 has the disadvantage that it is not orally bioavailable and currently no CD4 inhibitor has been approved for the clinic.

Ten years after the discovery of CD4 as primary receptor of HIV, the seven-transmembrane G protein-coupled chemokine receptors CCR5 and CXCR4 were discovered as critical coreceptors for HIV-1 entry [21–25]. Shortly thereafter, several groups reported that a homozygous deletion in the *CCR5* allele (*Δ32*/*Δ32*), naturally occurring in about 1% of the Caucasian population, protects against HIV-1 infection and is not associated with significant immunological dysfunction [26–28]. Moreover, it was observed that heterozygous deletions in *CCR5* are associated with delayed disease progression [27–29]. Altogether, these results suggested that CCR5 is a promising cellular target for anti-HIV therapy because its blockade should be well-tolerated and effective in inhibiting CCR5-tropic (R5) HIV-1 strains. Initially, several modified forms of the natural CCL5/RANTES (regulated upon activation, normal T cell expressed and secreted) ligand of CCR5 have been developed [30]. These agents compete with the HIV-1 gp120 for CCR5 binding and some of them (e.g. PSC-RANTES) are highly effective *in vitro* and in the human peripheral blood lymphocyte-severe combined immunodeficient (SCID) mouse model [30,31]. Although they failed in clinical trials, PSC-RANTES is currently further evaluated as potential microbicide against HIV [32,33]. Another strategy was the development of small molecule inhibitors that bind to a hydrophobic pocket in CCR5 and seem to inhibit HIV infection by inducing conformational changes rather than by direct occupation of the gp120 binding site [18]. One of these inhibitors, maraviroc, has been approved by the US Food and Drug Administration (FDA) and the European Medicine Agency for the treatment of viremic patients harboring multi-resistant HIV-1 strains. Thus, maraviroc is currently the only antiretroviral drug targeting a cellular factor used in the clinic [34]. However, several related agents, such as vicriviroc, and anti-CCR5 antibodies are currently evaluated in clinical trials [35,36].

An alternative approach is the specific knock-down of *CCR5* using RNA interference. Although this method usually only achieves partial knock-down of the target sequence, one study achieved the complete knock-out of CCR5 in hematopoietic stem cells using a stable RNA interference system, which then conferred resistance to HIV-1 infection to the *in vitro* derived macrophages [37]. More recently, the specific delivery of CCR5 siRNAs to T cells in a humanized mouse model was reported to suppress viremia and prevent CD4 T cell depletion [38]. Work is underway to reduce induced cytotoxicities to adapt siRNA-mediated knock-down of CCR5 for clinical application. These strategies have recently obtained substantial impetus by the spectacular report of a *Δ32*/*Δ32* allogenic stem cell transplantation in an HIV-1-positive leukemia patient, which led to long-term control of viral replication (for more than 2 years) in the absence of antiretroviral therapy [39]. Since HIV-1 usually only requires a few amino acid changes to switch from CCR5 to CXCR4 coreceptor usage [40], this is a highly unexpected and encouraging finding. The transplantation of CCR5-negative allogenic stem cells for the treatment of malignancies in HIV-1-positive patients holds some promise, particularly if the worldwide bone marrow and cord blood donor banks agree to merge CCR5-screening information into one database system [41]. A more general approach would be to knock-down CCR5 in wild-type CD34+ stem cells using RNA interference before transplantation. In order to be successful, this approach would have to allow the specific expansion of the transgenic cells *in vivo* since it is unlikely that gene transfer (of an siRNA expressing cassette) is achieved in all cells of the transplant. However, HIV-1 may facilitate this selection by causing a specific depletion of CCR5-expressing cells.

Although the inhibition or knock-down of CCR5 using small inhibitor molecules or gene therapy transfer protocols is promising, it also has some drawbacks. One concern is that CCR5 may play a role in immunity against some pathogens [42]. Another disadvantage is that it is not active against X4 HIV-1 strains. Obviously, the best approach would be to combine CCR5 and CXCR4 inhibitors [43]. However, in contrast to CCR5, CXCR4 is essential for various physiological processes and its knock-out is lethal in mice [44,45]. Several potent inhibitors of CXCR4, such as AMD3100 or AMD070, have been developed but clinical development was halted due to undesired side effects or lack of antiviral effects [1,18]. Notably, however, AMD3100 has been approved by the FDA as a stem cell mobilizer for transplantation under different names (Plerixafor or Mozobil). Several new anti-CXCR4 agents are in development and the CXCR4/SDF-1 pathway is emerging as an appealing target for the treatment of certain forms of cancer [46]. Thus, potent and better-tolerated CXCR4 inhibitors may become available in the future and complement antiretroviral therapies targeting CCR5.

2.2. Other cellular targets for antiretroviral therapy currently investigated

HIV-1 entry is a particularly promising step for intervention because it involves several relatively well-defined interactions in the cell membrane that can be blocked without the inhibitor entering the cells [47]. Furthermore, blocking HIV-1 at this early step prevents the integration of the proviral genome into that of the host cell and hence the establishment of latent viral reservoirs. However, the HIV-1 replication can in theory be inhibited at each step of its cycle in order to block viral spread. Thus, the inhibition of HIV-1 uncoating, reverse transcription, integration, transcription, assembly and release from the cell membrane (Fig. 1) all represent valid approaches to tackle HIV-1 infection and are currently being investigated.

An early therapeutic approach sought to target the HIV cofactors Tat and Rev, which are essential for viral replication and, to a large extent, functionally dependent on well-defined cellular factors [48]. Tat promotes the elongation of viral transcripts by binding to the transactivation response element (TAR) located in the HIV long-terminal repeat (LTR) and acts as an adaptor for the recruitment of the positive transcription elongation factor b (P-TEFb) [49], which is a heterodimer of cyclin T1 and cyclin-dependent kinase 9 (Cdk9). Since recruitment of P-TEFb to the TAR is both necessary and sufficient for HIV-1 transcription [50], targeting P-TEFb may constitute a good approach for anti-HIV therapy. A number of small compounds that inhibit Cdk9 or cyclin T1 activities, or that disrupt the Tat/TAR/P-TEFb interaction have been tested [51,52]. However, since P-TEFb is necessary for the transcription of many cellular genes [53], finding an inhibitor that exclusively blocks HIV transcription has proven difficult. Rev mediates the nuclear export of unspliced viral RNA through interaction with the *cis*-acting Rev response element (RRE) located in the HIV *env* gene [54,55]. The shuttling of Rev between the nucleus and the cytoplasm is dependent on a number of cellular proteins, including the RNA helicase DDX3X, CRM1, Ran-GTP as well as nucleoporins, importins and Sam 68 [54–56]. Targeting of DDX3X or other DEAD-box helicases by RNA interference has been reported to suppress Rev function and inhibit HIV replication [57–59]. Interestingly, the Tat cofactor CCNT1/cyclin T1 and the Rev cofactor DDX3X were identified in two of the three siRNA screens of host factors implicated in HIV-1 infection [7–9]. However, although Tat and Rev represent useful targets for antiretroviral drug development, no specific agents inhibiting them or their cellular cofactors without significant side effects have yet been developed.

The recent addition of an HIV-1 IN inhibitor (raltegravir) to the available HAART drugs indicates that targeting proviral integration is a useful approach. HIV-1 IN interacts with a cellular factor that may have potential as a therapeutic target: the cellular lens epithelium-derived growth factor (LEDGF)/p75 [60], a chromatin-associated protein that

is important for HIV-1 integration [61]. Disruption of the interaction between HIV-1 IN and LEDGF/p75 leads to impaired viral replication [62]. The crystal structure of the interaction interface has been resolved [63,64] and the development of a small inhibitor to preclude LEDGF/p75–IN binding is conceivable but remains challenging [65].

Although post-integration, the inhibition of particle budding from the plasma membrane and/or cell-to-cell transfer of the virus is a valid approach to prevent viral spread. HIV-1 usurps the cellular Endosomal Sorting Complex Required for Transport I (ESCRT-I) for its release from infected cells [66–68]. In particular, the PTAP-type late domain of the HIV-1 Gag precursor polyprotein interacts with TSG101 (tumor susceptibility gene 101), a cellular protein normally involved in endosomal protein sorting, and inhibition of this interaction or depletion of TSG101 by RNA interference suppresses HIV-1 particle release [69–71]. One therapeutic approach would consist in developing molecules, which would mimic the viral PTAP motif, such as cyclic peptides [72]. Another potentially interesting interaction has also been identified between HIV-1 Gag and the endosomal sorting protein Alix [73,74].

2.3. Genome-wide screens reveal numerous potential targets for antiretroviral therapy

Although many cellular factors were already known to be required for HIV infection, the recent application of technological advances to the study of HIV infection revealed that we were actually only seeing the tip of the iceberg. Three independent genome-wide RNA interference-based screens evaluated more than 20,000 human genes for their relevance in infection [7–9]. Altogether, these studies identified a total of 842 genes that reduce HIV-1 infection when knocked-down. Another study used an alternative approach and genotyped a large group of HIV-1-infected individuals to identify human genetic differences that influence the vulnerability to HIV-1 infection and clinical outcome of infection [75], thus adding a further 63 genes to the list. Recently, the National Library of Medicine made available a comprehensive list of all cellular proteins shown to interact physically or functionally with HIV-1 (Human Protein Interaction Database) [76–78]. Taken together, these studies and databases identified a total of 1,254 genes that may play a role in HIV-1 replication [79]. Many of these genes are involved in specific pathways (proteasomal targeting, transcription, immune response, RNA binding/splicing, chaperones, etc.) that are usurped by HIV-1 (summarized in Table 1).

The whole-genome siRNA screens, which expand the information brought by a previous subgenomic screen [80], provide important new insights on the host factors involved in HIV-1 early steps of replication. However, some caution must be taken in interpreting this newly available source of information. Firstly, the siRNA screens show only poor overlap: only three of the 842 HIV-dependency factors were obtained in all three studies: MED6, MED7 and RELA. The reason for this poor overlap lies possibly in differences in the cell lines used (HeLa and HEK293T), the multiplicity of siRNA coverage for each gene, the steps of the HIV replication cycle investigated, the time points analyzed and the filtering thresholds used [79]. Secondly, the use of cell lines (i.e. HeLa and HEK293T) that are not usually infected by HIV-1 and of pseudotyped virions limits the relevance of some of these results. Recently however, a genome-wide siRNA-based screen was carried out in Jurkats, thus identifying a further 252 genes involved in HIV-1 infection, only 6 of which were also identified by the 3 siRNA screens carried out in HeLa and HEK293T [81]. Thirdly, the siRNA screens focus per definition on proteins whose functions may be knocked-down without overt cytopathic effect, in other words, for proteins that are either functionally redundant or that are not essential for cellular function. Fourthly, it is likely that many hits represent false-positives in terms of physiological relevance since the studies only look at virus infection in cell lines as experimental endpoint and it is by no means certain that these play significant roles in HIV-1 infected individuals. These points, together with the partial knock-down nature of RNA interference, probably account for the fact that some cellular factors already known to be important for HIV-1 infection were missed by the genome-wide surveys (e.g. LEDGF/p75).

Despite some limitations, the genome-wide screens and surveys of HIV infection provide a good starting point for the identification of cellular targets for HIV therapy. Cellular factors that were identified in at least two of the three siRNA screens and are listed in the HIV interaction database represent particularly interesting candidates. Eleven proteins fulfill this criterion: the nucleoporin Nup153, CD4 and CXCR4, the kinases Jak1 and Akt1, the NFkB subunit RelA, four

Table 1
Selected host factors involved in HIV-1 infection.

Category	Example
Surface molecules	Clusters of differentiation; integrins; chemokines; MHC; lectins; syndecans; inositol triphosphate receptor
Endocytosis	Caveolin; clathrin; COPI system of vesicular transport
Intracellular receptors	Steroid receptors; nuclear receptors
Signaling	Ras family; tyrosine and serine/threonine kinases; phosphatases; cAMP phosphodiesterases
Cytoskeleton	Actin, microtubule, and intermediate filament components and associated proteins
Proteasomal degradation	Proteasome subunits; ubiquitin-conjugating enzymes, ligases and other associated proteins
Lysosomal degradation	Breakdown enzymes; vacuolar ATPases
Nuclear import	Nucleoporins; karyopherins; Ran binding proteins
Chromatin	Histones; histone clusters; histone deacetylases; chromatin modifying proteins; regulators of DNA repair/telomere length
Transcription & DNA binding	Transcription factors; co-activators; transcription elongation factors; RNA polymerases; mediator complex; DNA binding proteins
Nuclear export	RNA export factors; nuclear export signal dependent transport
RNA splicing	Splicing factors
Translation and RNA binding	Translation initiation factors; translation elongation factors; RNA helicases; ribonuclear proteins; ribosome-associated; protein ER translocation
Protein assembly/protein–protein interactions	Tripartite motif proteins; ankyrin repeat domains; heat shock proteins; chaperones
Protein modifications	Acetyltransferases; myristoyltransferases; peptidases; proteases
Metabolism	Isomerases; glycosyltransferases; convertases; phospholipases and other metabolic enzymes
Apoptosis	Inducers of apoptosis
Cell cycle & proliferation	Cyclins, cyclin-dependent kinases; cell division control proteins
Multivesicular body formation	ESCRT machinery; synaptic proteins
Cytokines and secreted proteins	Interleukins; tumor necrosis factor
Others	Antioxidants/metal binding; ion channels; complement

Shown are examples of host factors identified more than once in databases of host proteins whose knock-down attenuates HIV-1 infection, of single-nucleotide polymorphisms (SNPs) proposed to be associated with disease progression and of host proteins reported to interact with one or more viral component [7–9,75–79]. The list is not complete and the relevance of most of these cellular factors in primary HIV-1-infected cells remains to be confirmed.

cofactors for HIV-1 accessory and regulatory proteins and a keratan sulfotransferase of HIV-1 envelope [79]. Other interesting candidates are those appearing in three or more gene sets from a total of 12 that are currently available for factors identified as relevant for HIV replication [79]. In this case, 49 genes fulfill this criterion including Nup98, Nup153 and hnRNP1, all of which are thought to play a role in HIV nuclear import as well as TSG101 involved in virion assembly [79]. Proteasomal and lysosomal subunits, chaperones, heat shock proteins and transcription/translation factors also constitute multiple hits, but whether these are sufficiently important for HIV replication as to constitute a good drug target and can be selectively inhibited without cellular toxicity remains to be determined. These future studies and further improved RNAi-based screens have the potential to identify essentially all cellular cofactors required in the different steps of the HIV-1 life cycle and will undoubtedly not only greatly improve our understanding of host/HIV interactions but also lead to the development of new antiretroviral agents.

3. Host factors restricting HIV-1 replication

It is well appreciated that HIV uses many cellular factors to complete its life cycle. Only in recent years, however, has it become clear that the host cell is not a friendly environment for HIV since several host proteins have been identified as intrinsic immunity factors that most likely evolved specifically as defense against viral infections [10,11]. These host restriction factors interfere with retroviral replication by diverse mechanisms and can protect mammals from cross-species transmission of retroviruses. Three classes of retroviral restriction factors have so far been identified: cytidine deaminases (e.g. APOBEC3G), which induce lethal hypermutations of the retroviral genome [82,83], Fv1/TRIM5α proteins, which restrict the incoming retroviral capsid [84,85], and tetherin, which impedes the release of nascent HIV virions from the cell surface [14]. Recent analyses have shown that these host restriction factors all evolved under positive selective pressure due to past encounters with ancient viruses [86–89]. Overall, they were obviously quite successful since a large part of our genome (about 8%) consists of silenced retroviral sequences [16] and are still active against endogenous and exogenous invaders [90,91]. Notably, most restriction factors have broad antiviral activity and strengthening them may have beneficial effects against different pathogens [10,11,91].

APOBEC3G was the first host gene identified as an inhibitor of HIV-1 infection [13]. APOBEC3G is a cytidine deaminase that introduces G-to-A substitutions in the HIV-1 genome, which are detrimental to viral replication (Fig. 2). It has been reported that high levels of hypermutation in the provirus are associated with higher CD4 T cell counts in infected individuals [92], although not with reduced viral loads [93,94]. Accumulating evidence suggests that some of the antiretroviral activity of APOBEC3 is independent of its mutator activity and may involve direct effects on reverse

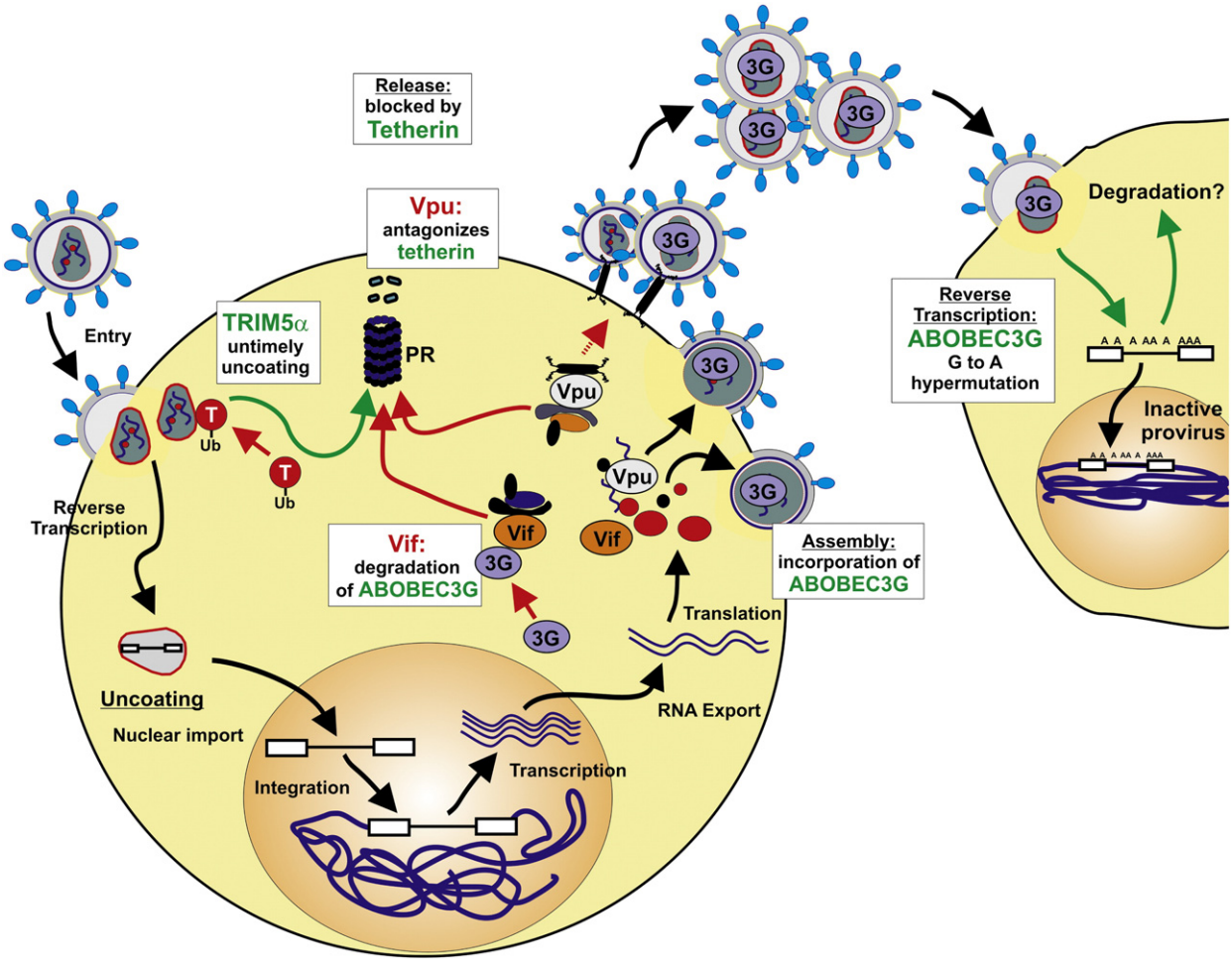

Fig. 2. Intrinsic host restriction factors and their viral antagonists. As schematically indicated, TRIM5α interacts with the incoming HIV-1 capsids and may induce accelerated uncoating by proteasomal degradation. The accessory viral Vif protein binds to a Cullin5-based ubiquitin ligase complex and to APOBEC3G (3G) to induce the degradation of the latter in proteasomes. In the absence of Vif, APOBEC3G is incorporated into the budding virions and causes lethal G-to-A hypermutations of the retroviral genome in the next round of infection. Tetherin prevents the release of nascent mature viral particles from the cell surface and is antagonized by Vpu. The exact mechanism by which Vpu counteracts tetherin remains to be identified but may involve direct interaction and beta-TrCP2-dependent degradation of tetherin leading to its sequestration from budding virions.

transcription or integration [11]. An increasing interest is attributed to the therapeutic inhibition of Vif, which antagonizes APOBEC3G by proteasome-mediated degradation and blocking its incorporation into nascent particles [95,96]. A different approach would be to boost the intracellular levels of APOBEC proteins through interferon-alpha treatment [97,98] or by targeting the Vif-APOBEC interaction domain. Alternatively, small molecules may be designed to enhance the catalytic activity of APOBEC3G in the cell. However, in order to consider APOBEC family members as potential targets for anti-HIV therapy, it will be important to clarify the relative contribution of the enzymatic and non-enzymatic activities of APOBEC to the observed restriction of viral replication and to elucidate whether these operate mainly in virions prior to infection or in the cytoplasm of infected cells.

TRIM5α proteins block retroviral infection of primate cells in a species-specific manner and were originally discovered as important determinants of the resistance of monkey cells to HIV-1 infection [12]. The mechanisms that lead to virus inactivation by TRIM5α proteins are not well understood [11]. It is thought, however, that the incoming viral capsids are rapidly uncoated upon entry into the cytoplasm of the host cell [99] and that the C-terminus of TRIM5α interacts directly with the viral capsid and determines its antiretroviral specificity [99,100]. HIV-1 is specifically blocked by TRIM5α from Rhesus macaques and Owl monkeys but only weakly restricted by human TRIM5α [84,85]. Therefore, the therapeutic targeting of TRIM5α for treatment of HIV-1 infection may be achieved by gene therapy mediated delivery of rhesus or owl monkey TRIM5α variants. Since these proteins are not human, a concern may be that modified cells would be eliminated by an immune response in treated patients. Furthermore, it remains to be elucidated whether HIV-1 can develop resistance against the monkey TRIM5α variants. Recently, chimeric forms of human–rhesus and human–owl TRIM5α were reported to restrict HIV-1 in transduced primary cells and in humanized mouse models [101,102], thus validating chimeric forms of TRIM5α as potential candidates for anti-HIV-1 gene therapy. Alternatively, it may be possible to develop compounds that promote the binding of TRIM5α to HIV-1 capsids or interact directly with the capsids to inactivate them. Finally, it is noteworthy that other members of the TRIM family, such as TRIM22, may have activity against HIV-1 [103,104]. Thus, the induction of some TRIM proteins may help to limit HIV-1 replication.

The most recently identified restriction factor, tetherin (also known as BST-2, CD317, or HM1.24), inhibits viral spread by "tethering" fully formed mature virions on infected cell surfaces and preventing them from budding [14,15]. Tetherin has broad antiviral activity: it inhibits a wide range of retroviruses as well as filo- and arenaviruses. Pandemic HIV-1 strains use their Vpu protein to antagonize tetherin [14,15,105]. In comparison, SIVs that lack a *vpu* gene counteract this restriction factor by their multi-functional accessory Negative factor (Nef) proteins [106,107] and HIV-2 and Ebola viruses seem to antagonize tetherin by their envelope glycoproteins [108–110]. The mechanisms by which these viral factors antagonize tetherin are not well understood but Vpu seems to sequester tetherin from the site of budding [14,110], reduce its surface expression [15] and promote its beta-TrCP2-dependent proteasomal degradation [111,112]. The expression of tetherin is inducible by interferon-alpha (IFNα) and high surface levels of tetherin suppress virus release even in the presence of Vpu [15,88]. Therefore, one possible therapeutic approach would be to enhance tetherin expression by treatment with IFNα. It has been reported that IFNα treatment in mice increases the levels of tetherin expression at the cell surface [113]. In early studies, recombinant IFNα has been used as a potential therapeutic for AIDS-associated Kaposi's sarcoma caused by HHV-8 and some patients showed reduced HIV plasma viremia [114]. Although IFNα levels are high in acute HIV-1 infection [115], evidence suggests that impaired type I interferon production is observed in AIDS patients [116]. A major problem with IFN-α treatment is that it has both beneficial effects – because it inhibits viral replication – but also harmful consequences as it contributes to the high levels of immune activation that drive progression to AIDS. Alternatively, tetherin expression and/or activity may be enhanced with the use of a compound drug. Interestingly, the cholesterol-binding compound inhibitor amphotericin B methyl ester (AME), previously shown to potently inhibit HIV-1 replication [117], was recently shown to interfere with the anti-tetherin function of Vpu [118]. Notably, recent data show that only the Vpu proteins of pandemic HIV-1 group M (major) but not of non-pandemic HIV-1 group O (outlier) strains efficiently antagonize tetherin [105]. Thus, efficient induction of tetherin may not only inhibit viral replication but potentially also reduce the rate of sexual transmission of HIV-1.

Accumulating data suggest that a number of restriction factors that interfere with primate lentiviral replication and are counteracted by other viral accessory genes remain to be identified [10,11]. For example, the HIV-1 Vpr and the HIV-2 Vpx proteins bind DCAF1 (VprBP) to engage the Cullin4 E3-ubiquitin ligase complex [119–121]. This interaction seems to be required for the ability of Vpx to antagonize an as-yet-unknown host restriction in human macrophages and dendritic cells [122,123]. Thus, the major function of the majority of the HIV accessory genes seems to be the antagonism of intrinsic immunity factors. However, HIV-1 also evolved sophisticated mechanisms to manipulate cellular trafficking, signal transduction and gene expression. In particular, the accessory viral Nef protein is well known for its ability to interact with a large variety of cellular factors in order to render the infected cell and their environment more conducive to viral replication and to facilitate viral immune evasion [10,11,124,125]. Intact *nef* genes are required for efficient HIV-1 replication in infected individuals and are associated with accelerated disease progression. Thus, agents disrupting the interaction of Nef with its cellular targets may have beneficial effects on the clinical course of infection.

4. Conclusions and future directions

Altogether the recent scientific advances demonstrate that the interaction between HIV-1 and its human host is far more complex than previously anticipated. The identification of numerous cellular factors that are exploited by HIV-1 at essentially every step of its replication cycle provides a large number of potential targets for antiretroviral therapy. However, a major challenge remains to separate the wheat from the chaff and important questions must be addressed before the bulk of this knowledge can be translated into clinical applications; e.g. which cellular factors are obligatory for HIV-1 replication in the relevant primary cell types *in vivo*?; which essential interactions between viral and host factors can be blocked without significant side effects?; which obligatory HIV-dependency factors can be knocked-out without important physiological consequences? Moreover, the identification of a potential target does not necessarily translate into the development of a therapeutic molecule. The "druggability" of a candidate protein [126] depends both on its propensity to be pharmacologically targeted — ideally an enzymatic domain whose endogenous binding partner can be out-competed by a small drug molecule — and on its ability to be efficiently delivered into target cells. While much work still remains to be done, further studies on the host proteins involved in HIV replication and their inhibition or elimination are highly warranted, particularly since virological and clinical analyses of the HIV-1-infected individual that received the Δ32/Δ32 allogenic stem cell transplantation provide proof-of-concept evidence that such strategies can achieve long-term control of viral replication in the absence of antiretroviral therapy [39,41].

It is conceivable that HIV-1 has evolved to efficiently antagonize those host defenses that are most relevant for its control. As discussed above, our current knowledge suggests that intrinsic host restriction

factors are usually quite effective against retroviruses. Thus, it will be interesting to further assess whether the efficient induction of these natural antiretroviral factors may overwhelm their viral antagonists and thus allow HIV-1-infected individuals to gain better control over viral replication. Finally, we should also consider that non-human primates naturally infected with SIV avoid disease progression not because they are able to efficiently control viral replication but because they show limited immune activation and preserved mucosal immunity [127,128]. Thus, not only strategies aiming to reduce viral replication but also alternative approaches to limit the excessive harmful levels of immune activation should be evaluated. Altogether, the recent scientific advances in our understanding of viral pathogenesis and on the cellular factors promoting or restricting HIV replication hold great promise for the development of improved treatment and prevention strategies.

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